Enrichment and characterization of high- and low-affinity herbicide-degrading bacteria from a groundwater aquifer

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ABSTRACT BOOK

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involves the cooperative metabolism of syntrophic bacteria, which catalyze the initial attack and subsequent degradation of hydrocarbons, and methanogens, which convert intermediates such as hydrogen, formate, carbon dioxide and acetate to methane. The identity of syntrophic microbes and the nature of their interactions with other syntrophs and methanogens are not well understood. Furthermore, it is difficult to isolate the organisms responsible for the initial activation and subsequent degradation of hydrocarbon substrates under methanogenic conditions due to the thermodynamic relationships which exist among microbes in methanogenic communities. In order to identify the organisms capable of the activation and subsequent degradation of toluene under methanogenic conditions, we have carried out RNA-SIP on an established methanogenic toluene degrading enrichment culture. RNA was extracted from microcosms amended with either $^{13}C_7$-toluene or $^{12}C_7$-toluene in a time course experiment. Our results indicate that ^{13}C-carbon was initially assimilated by an organism belonging to the Clostridiaceae and an organism belonging to the Deltaproteobacteria. ^{13}C-carbon was later assimilated by organisms related to the Desulfovibrionales and Spirochaetales. Our results indicate that the initial attack on toluene in this culture is mediated by organisms belonging to the Deltaproteobacteria and/or the Clostridiaceae. Analysis of metagenomic contigs from this culture containing genes involved in toluene metabolism have provided further insight into the origin of these genes and the possibility of horizontal gene transfer within this culture. The discovery of the involvement of members of the Clostridiaceae in toluene metabolism is a novel finding. Previous gram-positive organisms that have been shown to be involved in aromatic hydrocarbon metabolism have grouped within the Peptococcaceae. The involvement of Deltaproteobacteria in aromatic hydrocarbon metabolism has been described previously, but not under methanogenic conditions.

237B In *Caulobacter crescentus*, the artificial production of the alarmone (p)ppGpp retards cell differenciation and initiation of chromosome replication

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In most bacteria, starvation triggers, via the alarmone (p)ppGpp, dramatic changes in metabolic functions, but also modifications in the cell cycle. *Caulobacter crescentus* has a two-stage life history, presenting a juvenile motile form, the swarmer cell, that has to differentiate into a reproductive sessile form, the stalked cell, before replicating its DNA and dividing asymmetrically. The genome of *C. crescentus* encodes a (p)ppGpp synthetase, that is required to delay the initiation of DNA replication under conditions of carbon starvation. By contrast, the influence of (p)ppGpp accumulation on the differentiation process has not been convincingly assessed yet.

Here we report the construction of a *C. crescentus* strain in which a stringent response can be induced in the absence of actual starvation by the expression of the gene *relA',* coding for a constitutively active form of RelA, a (p)ppGpp synthetase from *Escherichia coli.* By studying synchronized populations, we observed that the expression of RelA' is sufficient to significantly retard the replisome assembly and the initiation of DNA replication, most likely by stimulating the proteolysis of DnaA. Furthermore, using fluorescently labeled proteins that localize at different stages of the cell cycle, we could demonstrate that important molecular events characteristic of the swarmer-to-stalked cell transition are delayed as well when RelA' is induced. We propose that, in stringent conditions, the intracellular accumulation of (p)ppGpp could favor dispersal and foraging functions over multiplication by extending the lifespan of the motile, non-reproductive swarmer cell, and thus play a major role in the ecological adaptation of *C. crescentus* to nutritional stresses.

238B Enrichment and characterization of high- and low-affinity herbicide-degrading bacteria from a groundwater aquifer

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Enrichment culturing has long been the method of choice for selecting and isolating catabolic microorganisms, and it has been used successfully to obtain bacteria capable of degrading xenobiotic

compounds. However, standard enrichment cultures using high pollutant concentrations may have overlooked populations existing in the same micro-niches that are adapted to metabolizing substrates at low concentrations. These populations could be more efficient at remediating low levels of organic pollutants in contaminated water resources. Thus, comparing high- and low-affinity enrichments could facilitate the development of new and more efficient approaches for remediation of pollutants at low concentrations. In this study we compare the mineralization and cell growth dynamics, as well as changes in bacterial community structure, of two enrichment cultures that were selected from a groundwater aquifer on low (100 µg L^{-1}) or high (25 mg L^{-1}) concentrations of the herbicide 4-chloro-2-methylphenoxyacetic acid (MCPA). MCPA is a phenoxyalkanoic acid herbicide used worldwide to control broad leaf weeds and has been introduced into the environment in large amounts.

Saturated sediment samples were collected from a Danish groundwater aquifer. The MCPA mineralization by the bacterial cultures was quantified by radiorespirometry using ^{14}C ring-labeled MCPA, and growth of the cultures and single cell physiology were analysed by flow cytometry. The structure and composition of the cultures at appropriate time points of extensive mineralization activity were analysed by 16S rRNA gene denaturing gradient gel electrophoresis fingerprinting and subsequently more in depth by pyrosequencing.

We show that different populations of herbicide-degrading bacteria adapted to metabolizing substrates at different concentrations can co-exist in a community and can be selected using conventional enrichment techniques. These populations showed differences in metabolic efficiency and community composition as well as physiology at the single-cell level. Furthermore, the enrichments selected on low concentrations were more efficient at mineralizing MCPA regardless of concentration. The proliferation of low nucleic acid-bacteria linked to metabolic activity and major changes in community composition in cultures selected on low herbicide concentration suggest that this group of bacteria could play a role in degradation of low herbicide concentrations in groundwater aquifers impacted by agricultural practice.

Our findings suggest that using environmentally relevant substrate concentrations, in contrast to the standard high concentrations, could provide bacterial communities that are more efficient in terms of metabolic functionality and stability when degradation of low pollutant concentrations is desired.

240B Microbial response of dedicated pesticide degraders to low and poorly bio-available concentrations of pesticides

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The widely use of pesticides in agricultural for minimising crop yield losses results into their dissemination into the environment and contamination of soil, groundwater and surface water. Biodegradation by specialized adapted organisms is seen as one of the major routes for pesticide degradation. Dissemination and biodegradation of organic compounds mainly depends on the pesticides´ bioavailability and hence on the pesticides' properties concerning their distribution between liquid and solid phase. Water soluble compounds are quickly diluted and spread over large areas in surface and ground water bodies while more hydrophobic compounds sorb to soil particles or to suspended solids in the aqueous phase. As a result, pesticides usually occur in low concentrations in the environment. Hence, microbial degradation of these compounds and the formation of non-toxic products (CO_2 and H_2O) mainly depend on the capability of microorganisms to mineralise low concentrations and/or their ability to solubilise soil or suspended solid bound fractions. In this study, we examined how sorption affects the mineralization of the relatively strongly sorbing pesticide linuron, the moderately sorbing herbicide carbofuran and the highly water soluble pesticide metabolite 2,6-dichlorobenzamide (BAM). Mineralisation of low concentrations (17 µg/l to 500 µg/l, using ^{14}C-labelled compounds) were tested with the strains *Variovorax* sp. SRS16 (linuron degrader), *Sphingomonas* sp. KN65.2 (carbofuran degrader) and *Aminobacter* sp. MSH1 (BAM degrader). The strains´ capacity to mobilise sorbed pesticides were investigated using the adsorber resin XAD-7HP in a medium-adsorber ratio resulting in 75%, 95% or 99% pesticide adsorption. Mineralisation characteristics of the three different strains differed distinctly. KN65.2 mineralised the different concentrations of carbofuran